# CALIFORNIA INSTITUTE OF TECHNOLOGY Division of Biology Pasadena, California

# PROGRESS REPORT

on

Neural Control of Hibernation in Mammals; the Relevance of Physiological Clocks and Environmental Factors

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This progress report covering the period September 1, 1965 - February 28, 1966 includes three papers:

- Parameter selection for periodicity measurement by the method of power spectral analysis by Floyd R. Schlechte.
- Processing and analyzing hibernation data recorded on digital magnetic tape by John A. Streeter.
- III. Prolonged in vitro culture and autoradiographic studies of neurons in Aplysia by Felix Strumwasser and Renate Bahr.

# PARAMETER SELECTION FOR PERIODICITY MEASUREMENT BY THE METHOD OF POWER SPECTRAL ANALYSIS

by Floyd R. Schlechte

#### SUMMARY

The equations of power spectral analysis were used to estimate periodicities in both computer-generated and real data. The real data were brain temperatures recorded from squirrels of a hibernating species. From a study of the calculated results rules of thumb were developed for specifying the data grouping interval, the number of data groups, and the maximum lag in the autocorrelation coefficients for input to the power spectra.

# I. INTRODUCTION

In order to perform any autocorrelation and power spectral analysis on discrete data it is necessary to choose three elementary parameters: the data grouping interval, the number of data groups, and the maximum autocorrelation lag for input to the power spectra. The effect of alternative choices of these quantities on calculated periods in actual data by this method is very difficult if not impossible to find in the literature. All the journals indicated as references 1 through 5 were searched.

A series of calculations was therefore performed in order to put the selection of parameters on a rational basis. The effect of the maximum autocorrelation lag was studied using an exact expression for the power spectra of a continuous sine wave. The effect of noise was included by using a computer-generated discrete sine wave with random noise from a rectangular distribution of numbers from Reference 6. Through the use of actual brain temperature data from a squirrel, Citellus lateralis (#2), the effect of the size of the data grouping interval relative to period size was studied. From the calculated results came a set of suggested rules of thumb which are probably better than wild guesses. However it is probably still true that in almost every case where periodicities are to be extracted from data by the method of power spectral analysis some experimentation with parameters will be required.

## II. THE GENERATION OF A DISCRETE-DATA SINE WAVE PLUS NOISE

A computer program was written to generate data suitable for a sine-wave-plus-noise analysis. The program requires that the number of points per cycle of sine wave and the nominal ratio of RMS noise to RMS signal be specified. For each point on the sine wave a random number was chosen from a random rectangular distribution (Ref. 5). As a check on the noise level the exact ratios of RMS noise to RMS signal were calculated

from the numbers actually used. The nominal and exact ratios were usually the same within about 1% and the nominal ratios appear in the tables.

# III. THE AUTOCORRELATION AND POWER SPECTRA OF DISCRETE DATA

For the autocorrelograms the average of the raw data was subtracted first. Then the autocorrelation coefficients were calculated. Finally these coefficients were normalized with respect to the coefficient at lag zero.

The autocorrelation coefficients were used as input to a power spectra program and the period was estimated after calculating extra ordinates in the power spectra in the neighborhood of a peak. The power spectra program covered in the previous progress report was used.

# IV. AN EXACT ANALYSIS FOR A CONTINUOUS SINE WAVE AND A NUMERICAL ANALYSIS FOR NOISELESS DISCRETE DATA

The period of a continuous sine wave was calculated by locating the peak in the exact expression for its power spectra. The autocorrelation used as input to the power spectra was assumed to be a pure cosine. The maximum lag in the autocorrelation was considered a variable parameter. Results are shown in Table 1 for a few values of maximum lag near 3, 7, and 14 cycles. Similar results are shown for discrete data analyses in Tables 2 and 3a. The maximum error due to the finite number of cycles of lags is about one part in 100, one part in 1000, and one part in 5000 for the values of maximum lag mentioned above.

# V. ANALYSIS OF NOISY DISCRETE DATA

Examples of periodicity estimates based on interpolated power spectra of a computer-generated sine wave plus noise are given in Tables 3b and 3c. The results show that with a maximum lag of 2.83 cycles and an RMS noise to signal ratio less than 1 the error in the period due to noise can be 1 part in 200. The 2.83 cycles is about 10% of the data duration. When the maximum lag is 14 cycles and the noise to signal ratio is less than 1 the error due to noise is about one in 1200. In the latter case, if the noise ratio is raised to 2.5 the error in period estimate increases to about one in 350. The effect of noise on the autocorrelogram peak in the neighborhood of a one-cycle lag is shown in Table 3c.

Twenty-four weeks of the brain temperature of a golden-mantled squirrel, Citellus lateralis (#2) was analyzed by autocorrelation and power spectra. Three sets of calculations of circadian period were made using different choices of parameters. The results are shown in Table 4, columns a, b, and c. Column a is based on a maximum lag of 2.8 days and 48 points per day. Column b is based on a maximum lag of 14 cycles and 48 data points per day. Column c is based on a maximum lag of 14 cycles and 8 data points

per day. The period estimates show that the shift in maximum lag had more effect than the shift in the number of points per day. In Table 4, columns d and e, the autocorrelogram peaks in the region near a lag of 24 hours are given for both values of sampling interval. The noise level shows considerable variation from month to month. The best agreement between indicated periods in columns b and c occurs with the block of data showing the strongest periodicity in the autocorrelogram (4-26-65 to 5-23-65). The larger sampling interval reduced the noise component of the autocorrelogram.

In the calculations shown so far the sampling interval has not been coarse enough to affect the indicated period as determined from the interpolated power spectra. Table 5 shows an extension of Table 4 to 12-hour and 8-hour periods. The variation in indicated period is more sensitive to the data grouping interval here than for the circadian rhythm. Probably the low magnitude of oscillation rather than the cut-off in frequency resolution is the major cause of the differences. But a grouping interval of 3/8 of a cycle, as in the case of the 8-hour period, is probably too coarse. A number of writers, including Blackman and Tukey in Reference 7, recommend that the sampling interval be small enough to provide at least 3 data points per period and this may be a good rule.

# VI. DISCUSSION

Blackman and Tukey in Reference 7 recommend that the maximum lag be kept under 10% of the test duration to stabilize the estimates of power spectra ordinates. Intuition would seem to indicate that the same procedure should give the best estimate of frequency at the spectral peak. In the present study the power spectra formula was used to interpolate between the ordinary discrete ordinates to search for a peak. Calculations using as input a sine wave plus noise have consistently yielded better measures of signal frequency when the maximum lag was significantly increased. It seems best to use as great a maximum lag as can be used, at least 10 to 14 cycles. But the optimum maximum lag in a practical case is dependent on the rate at which the period is changing. The period being measured should be constant for the test duration.

#### IN BRIEF

If the problem is to isolate periodicities in discrete data by the method of interpolated power spectra then the basic parameters may be chosen as follows:

- a) Put at least three data groups in the time corresponding to the length of period being studied.
- b) Use at least 10 to 14 cycles of lags as input to the power spectra.
- c) When calculating autocorrelation coefficients keep the maximum lag under half the duration of the data being analyzed.

Table 1. Period of a continuous sine wave of infinite length calculated from the analytical formula for the power spectra. True period was 24 hours.

Estimated period, hours	Magnitude of error, per cent
23.856	0.602
24.004	0.016
24.122	0.504
23.998	0.008
23.979	0.089
24.000	0.001
24.020	0.084
24.000	0.000
23 <b>.995</b>	0.020
24.000	0.001
24.005	0.021
24.000	0.000
	period, hours  23.856 24.004 24.122 23.998  23.979 24.000 24.020 24.020 24.000 23.995 24.000 24.000

Table 2. Periods calculated by interpolated power spectra of computer-generated sine wave. 14 cycles of data. 48 points/cycle. True period, 24 hours. Variable maximum lag

Maximum lag, cycles	Estimated period, hours
0.96 1.00 1.08 1.21 1.33 1.46 1.58 1.71 1.83	23.08 23.12 23.64 24.49 24.35 23.62 23.82 24.24 24.18 23.79
2.96	23.91
3.96	23.95
5.96	23.98
6.00	23.97
6.08	23.99
6.21	24.02
6.33	24.01
6.46	23.98
6.58	23.99
6.71	24.02
6.83	24.01
6.96	23.98

Table 3. Periods calculated by interpolated power spectra of computer-generated sine wave plus noise. 28 cycles of data. 12 points/cycle. True period, 24 hours.

# a. Variable maximum lag. Zero noise.

Maximum lag, cycles	Estimated period, hours		
2.17	24.02		
2.33	24.23		
2.50	23.85		
2.67	24.03		
2.83	24.21		
14.00	23.999		

# b. Variable noise. Maximum lag, 2.83 cycles.

RMS noise	Estimated		
RMS signal	period, hours		
0.0	24.21		
.2	24.12		
.4	24.15		
1.0	24.20		

# c. Variable noise. Maximum lag, 14.0 cycles.

RMS noise RMS signal	Estimated period, hours	Autocorrelation peak at lag of l cycle
0.0	23•999	1.00
.2	23.995	•97
.4	24.009	.86
1.0	23.980	•47
1.5	23.996	.31
2.0	23.956	.29
2.5	24.067	.12

Table 4. Circadian periods calculated by interpolated power spectra of continuously recorded brain temperature of a squirrel, <u>Citellus lateralis</u> (#2). Each batch of data covers 28 days.

- a) Data grouping interval and lag step = 0.5 hour.
  Maximum lag = 2.8 days.
- b) Data grouping interval and lag = 0.5 hour. Maximum lag = 14.0 days.
- c) Data grouping interval and lag step = 3.0 hours.

  Maximum lag = 14.0 days.
- d) Ordinate of autocorrelogram at peak where lag is about 24 hours and a 0.5 hour sampling interval was used.
- e) Similar to d except a 3.0 hour sampling interval was used.

Inclusive dates		Estimat	Estimated period, hours			Autocorrelation peak at lag of about 24 hours	
		(a)	(b)	(c)	(d)	(e)	
3-1-65 to	3-28-65	24.08	23.98	23 <b>.97</b>	0.26	0.36	
3-29-65	4-25-65	24.13	23.78	23.79	0.39	0.47	
4-26-65	5-23-65	24.16	24.02	24.02	0.56	0.71	
5-24-65	6-20-65	24.09	23.92	23.91	0.33	0.44	
6-21-65	7-18-65	24.80	24.14	24.13	0.21	0.27	
7-19-65	8-15-65	23.85	23.96	23.95	0.40	0.49	

Table 5. 8-hour and 12-hour periods for same data referred to in Table 4. Maximum lag = 14 days.

- a) Data grouping interval and lag step = 0.5 hour.
- b) Data grouping interval and lag step = 3.0 hours.

Inclusiv	e dates	(a)	timated pe (b)	eriod, hour (a)	s (b)
3- 1-65 t	o 3-28 <b>-6</b> 5	7.9947	8.104	12.018	12.027
3-29-65	4-25-65	8.0148	7.990	12.007	12.017
4-26-65	5-23-65	8.1020	8.095	11.849	11.818
5-24-65	6-20-65	7.9978	7.968	12.235	12.219
6-21-65	7-18-65	7.9728	7.983	11.995	11.988
7-19-65	8-15-65	7.9764	7.976	11.973	11.968

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# PROCESSING AND ANALYZING HIBERNATION DATA RECORDED ON DIGITAL MAGNETIC TAPE

by John A. Streeter

N66 28748

### I. GENERAL PURPOSES

This report is a follow-up of the data acquisition program described in the previous progress report (September, 1965). A brief description of that program is first included to orient the reader.

Since that report, entitled "A Program to Compress, Reformat, and Summarize the Magnetic Tape Record of Several Intermixed Time Series," several difficulties in processing the data have arisen. It is hoped that a discussion of these difficulties and their solutions will be of value to investigators who are interested in using a similar method of data collection.

This report will also contain a description of a program to check, edit, and combine tapes used for data storage.

Tape processing expenses will be summarized on a monthly (4 week) basis.

Last, some of the graphical and statistical techniques used in analyzing hibernation data will be described.

# II. A BRIEF DESCRIPTION OF THE DATA ACQUISITION PROCESS

The data for this hibernation study consists primarily of a digital magnetic tape record of the brain temperatures of several hibernators (ground squirrels). Environmental temperatures and calibration checks are also recorded on the same tape. Brain temperature is read by a digital voltmeter from a thermocouple implanted in the brain of each squirrel. The time (in hours, minutes, and seconds) and an identifying channel number are recorded with each reading.

This method of monitoring has produced a record of brain and environmental temperatures which is virtually uninterrupted for 14 months. With the present equipment, up to 7 data channels are being scanned sequentially, 24 hours a day at the rate of one channel every 10 seconds. The system is capable of scanning 12 channels at twice that rate as well as scanning 7 counters every 5 minutes.

III. PROBLEMS ASSOCIATED WITH THE PROGRAM TO 'COMPRESS, REFORMAT, AND SUMMARIZE' THE MAGNETIC TAPE RECORD.

During the first 8 months of continuous round-the-clock recording many additions were made to the original program. These additions corrected

unexpected, but usually systematic, errors in the data collection equipment. Such errors consisted of omissions in the scanning sequence, failure of a counter to reset, recording a phantom channel, etc.

These errors occurred with a fair degree of regularity. They were frequent, but predictable, and hence not difficult to correct by such means as checks on the time sequence, the channel number sequence, the range of the readings, or the range of the first differences.

Illegible tapes began to occur during the last 4 months of 1965. Errors associated with illegible spots on the tape were more difficult to eliminate—they were often random, and sometimes plausible. In some cases, the tape was physically impaired because of mechanical problems with the tape transport. In other cases, the trouble lay in the electronics of the equipment. Incomplete words, incomplete records, or meaningless bit configurations were recorded. Often thousands of illegible records were interspersed among thousands of good ones.

Attempts to read these tapes under the standard IBM input-output control system (IOCS) for the IBM 7094 resulted in incomplete runs with a total loss of any punched card output. Under IOCS the computer would attempt to read and re-read the bad portion several times. Each attempt was accompanied by an on-line message to the operator indicating that a "permanent read redundancy" existed or that a "noise record" had been discarded. Each on-line message took approximately 1 second of computing time, so it was not possible to allow hundreds of messages to be printed. Frequently the tape would not advance no matter how many messages were printed.

The problem was presented to several members of the Computing Center staff and to the IBM operators and customer engineers who worked there. As an initial remedy it was suggested that the tape be routinely submitted a second time in case dust or tape misalignment were the cause. This method was costly and rarely worked. It was soon abandoned.

The next solution was to attempt to copy the tape. Several standard tape copying programs were available. In practice, however, they usually required the manual intervention of the operator to get the tape past each severely illegible portion. This work had to be done during the early morning shift when the computer was not heavily scheduled. At times the frequency of the illegible spots required an extreme number of manual interventions, making this solution inconvenient for the operators. This copying was done as a peripheral operation with no charge to the user.

A suggestion was made by the IBM customer engineer that a routine be written which would copy the tape without using the standard IOCS for tape handling. He was skeptical about incorporating such a routine into the present processing program so that the tape would not have to be copied at all.

A request was placed with the Caltech Computing Center staff to produce a tape reading routine which contained no reading checks. The

staff member in charge of IOCS suggested it might be easier to remove the checks from the existing IOCS. Although initially skeptical about the feasibility of such changes to IOCS, he soon produced a small XMAP patching routine which was simple and effective. Since the time it was incorporated into the processing routine, no program has been interrupted because of inability to read the tape.

When reading imperfect tapes, one frequently encounters "garbaged" records. For instance, omitted words often cause a time reading to be in the place of a voltage reading and vice versa. Frequently the digits in a word will be shifted to the right or left. Under the modified IOCS such records are permitted to enter the computer memory. As a result, many additional time sequence checks have had to be added. Such checks have to "look ahead" on the tape to see that a bad reading is not being interpreted as a plausible change in time.

When recognized, erroneous readings should be ignored and processing should continue from the next good reading. Error messages should be printed as each error is encountered. If the error is frequent, the number of such messages must be controlled to avoid printing hundreds of pages of error messages.

# IV. HANDLING THE COMPRESSED TAPE RECORD OF THE RAW DATA

For reasons of simplicity and reliability the raw data is recorded on tape in the lab at a low density (200 cpi) in BCD format. A 10-1/2 inch reel of raw readings can hold about 2 weeks of data, recorded at the scanning rate of one channel every 10 seconds. Such a tape contains about 25,000 records, where each record represents one complete sequential scan of all the selected channels.

For many reasons the format of the raw tape makes it unsuitable as a means of permanently storing the data. Each tape contains only 2 weeks of data. About 50% of the tape consists of record gaps. The recording density is low. Readings from the same channel are not grouped in any way. The readings are not edited, and the bit configuration must be changed from BCD to binary before the readings can be used in arithmetic operations.

The routine discussed in the previous section produces an edited, high density, binary tape in which all the good readings are binarized and arranged chronologically in hourly records by ascending channel number. This tape is called the compressed tape. One 10-1/2 inch reel will hold 10 to 12 weeks of data. Hence the compressed tape has 5 to 6 times the storage capacity of the raw tape, and an equivalent volume of data can be read into the computer 5 to 6 times as fast.

Because of the cost involved in reading or writing near the end of a tape, only 4 weeks of data are being written on compressed tapes. If a raw tape is in very bad shape and has to be submitted several times, it is more economical to start writing on a new tape than to risk the expense of having to position the last compressed tape past the old data several times.

Eventually the number of tapes will become unreasonable. A program has been written to combine several compressed tapes into one permanent storage tape containing 8 weeks of data. The format remains the same; however, since 5-minute and hourly summaries of the data are available from the initial processing, a closer editing of the tape record is possible. As the compressed tapes are being combined into one storage tape the temporal sequence is checked and close tolerance checks on the data and/or the first differences are performed. This final tape is checked and copied by the same program. It is then thought safe for all previous raw and compressed tapes to be erased and reused.

#### V. PROCESSING EXPENSES

The computer charges at Caltech are 5 dollars per minute. This rate applies to compiler time, assembler time, loader time, and system time as well as execution time. No charge is made for the off-line operations of printing and plotting.

It takes the 7094 tape drives about 4 to 5 minutes to read to the end of a 10-1/2 inch reel of tape, regardless of format. Because of the computer's buffering ability, a considerable number of calculations can be made during the time the tape is being read. The computer has two tape handling channels so that a compressed tape can be written as the raw data tape is being read, with only a small (less than 20%) increase in execution time.

Execution time for processing one reel (2 weeks) of raw data is between 6 and 6-1/2 minutes. Charges for items other than execution are significant. 10 to 15 seconds of computer time are used to mount the tape. During heavily scheduled periods, this time may increase. Up to 30 seconds are required to load large programs into memory from disc storage and/or binary card decks. A minimum of 10 seconds compiler and assembler time is required each time a subroutine is altered.

A minimum monthly (4 week) cost for processing the raw data would include the following time charges:

Processing 2 reels of raw data

15 minutes

Editing and producing a storage tape

4 minutes

Checking the 5-minute punched card averages for omissions or cards out of sequence and producing a printed tabular record of the averages

3 minutes

22 minutes

In practice, raw data tapes have had to be submitted more than once because of illegible spots on the tapes. At other times the tape would have to be resubmitted because a new kind of error had arisen which required a program modification for its elimination. These resubmissions often doubled the processing cost. There have been no resubmissions because of illegible tapes since the patch to IOCS has been included. Because tape handling is so expensive, all the statistical and graphical analyses of the data have been done from punched cards containing 5-minute averages. These cards are produced during the processing of the raw data tape. Although bulky and inapplicable where a finer resolution than 5 minutes is required, the increase in flexibility and decrease in operating expenses have made these cards indispensible.

An evaluation of the cost versus the usefulness of a storage tape containing every good reading is being planned.

# VI. SOME GRAPHICAL AND STATISTICAL TECHNIQUES USED TO STUDY HIBERNATION

The data has been graphed in several forms to show the following characteristics of the temperature record of hibernators:

- 1) Periodicities of time lengths from hours to months.
- 2) The smoothness or roughness of the animal's own temperature regulating mechanism at various stages in the hibernation cycle.
- 3) Peculiar peaks or valleys indicative of the onset of a new phase in the hibernation cycle.
- 4) The time, slope, duration, and range of temperature during entrance into hibernation and arousal from hibernation.
- 5) The duration and depth of hibernation.
- 6) Long term changes in mean temperature denoting changes in the health or seasonal state of the animal.

As the computer produces the graphical representation of the data, many of the above characteristics are quantized. For instance, the mean standard deviation, range, maximum point, and minimum point can indicate how smoothly the temperature was regulated, when the highest or lowest temperature occurred, or where a behavioral change is reflected in the record by a change in mean temperature.

Transformations of the data are frequently necessary before graphing. Periodicities can sometimes be shown more clearly if the data is smoothed before being graphed. If the length of the period is known or hypothetically determined, the "average shape" of one cycle can be seen by summing corresponding points over many cycles. Several graphing programs have been written which can perform from one to all of the following operations:

- 1) Average several readings together or sample the readings in order to lessen the number of points to be graphed.
- 2) Smooth the data by taking moving averages.
- 3) Linearly transform the data.
- 4) Sum corresponding points over many cycles of a predetermined period.
- 5) Perform low or high order interpolation where gaps in the data are present.
- 6) Calculate the mean standard deviation and range of the data.
- 7) Give the maximum and minimum ordinate values and the times at which they occur.
- 8) Produce more than one curve per graph sheet. The scale settings for each curve are arbitrary.

A program is being tested now to have the computer recognize and give the times of entrance and arousal from hibernation. This program was written by an undergraduate, LeRoy Nelson, as part of an honors project. It will eliminate the errors of human judgment which are now a part of phase alignment in graphing successive hibernations.

The curves produced by the computer are graphed by a Moseley plotter on 10 x 15 inch graph sheets. The graphs are suitable for photographing. A fade-out light-blue grid is most commonly used. Each curve usually involves some data transformation, and if the points are widely spaced, interpolated values must be provided or errors will be introduced by the momentum of the stylus. Each curve uses from 3 to 4 seconds of computer time. Over 200 curves of the hibernation data have been plotted so far.

Periodicities have been studied using the statistical techniques of periodograms, autocorrelograms, and power spectral estimates. Periodograms were computed to test for periodicities in the neighborhood of 24 hours. Such a periodicity was clearly established by this technique. However, because the periodogram function cannot be used for power spectral estimates, most periodicities have been determined by the autocorrelation function from which the dominant periods and their relative strengths can be determined to a higher degree of resolution by applying power spectral techniques. The ordinates of the periodogram at trial periods of interest are useful normalized figures for comparing the strengths of periodicities in different sets of data. The procedure for calculating periodograms is found in Whittaker and Robinson's book, The Calculus of Observations (Blackie & Son, Ltd., 1940).

Except for the periodograms, Mr. Floyd R. Schlechte has handled all the calculations of periodicities. He describes his programs and their use in another section of this report.

PROLONGED IN VITRO CULTURE AND AUTORADIOGRAPHIC STUDIES OF NEURONS IN APLYSIA. Felix Strumwasser and Renate Bahr.\* Calif. Inst. Technology, Pasadena, Calif.

Further studies have been undertaken to understand the mechanism of circadian oscillation in PB, and its entrainment by environmental photoperiod ("Circadian Clocks," 442-462, '65). In vitro culture of the isolated abdominal ganglion has been accomplished, for periods up to 6 weeks, using Eagle's minimum essential medium made up in sea water containing 20% Aplysia blood and antibiotics. Normal resting and action potentials and repetitive firing in response to applied trans-membrane current are present in identifiable neurons. Up to 3 weeks, cells with particular patterns of impulse activity still maintain these patterns, indicating that input through sensory, hormonal and central channels are not relevant for their genesis. The majority of output axons in the trunks are excitable allowing antidromic invasion. Afferents in the trunk are also excitable and monosynaptic connections show little sign of degeneration by 3 weeks. Autoradiographs on frozen and paraffin sections of fresh ganglia indicate that tritiated leucine is incorporated into protein in the cytoplasm and tritiated uridine into RNA in the nucleus of neurons. The patterns of synthesis in adjacent neurons may not be uniform indicating that neurons can be in different states, perhaps an indication of the electrical output that they are engaged in.

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